Atty. Dkt. No. 081356-0262

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Nobuaki TAKAHASHI *et al.*

Title: **ANTI-CD40 ANTIBODY MUTANTS**

Appl. No.: 10/584,345

Examiner: Phillip GAMBEL

Art Unit: 1644

Confirmation Number: 3671

DECLARATION OF DR. NOBUAKI TAKAHASHI UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Nobuaki Takahashi, hereby declare and state as follows:

1. I currently hold the position of Senior Scientist in the Antibody Research Laboratories Research Division of KYOWA HAKKO KIRIN CO., LTD. ("KHK"), Japan, the assignee of the captioned application.

2. I have been employed by KHK since 1995, when I obtained my Ph.D. from Osaka University. I worked at Gemini Science, Inc., a subsidiary of KHK in the U.S., from 1998 to 2000. In that position I generated and evaluated various monoclonal antibodies. Since that time I have worked on the research and development of monoclonal antibodies.

3. I am a co-inventor of the captioned application, in relation to which I have submitted a previous declaration. It is expected that I would be remunerated when KHK gains income from commercializing a product or technology that is related to the invention of the application.

4. I understand that the application is directed to an antagonistic CD40 antibody designated "4D11G4PE;" compositions that comprise the 4D11G4PE antibody; and a method of treating or preventing transplant rejection by administration of the 4D11G4PE antibody.

5. The 4D11G4PE antibody, a mutant of the 4D11 antibody, is an anti-CD40 antagonistic antibody. Relative to the 4D11 antibody, the 4D11G4PE antibody contains two amino acid substitutions at positions S228P and L235E, as indicated by the EU index of Kabat *et al.*, a standard antibody notation system.

6. While employed with KHK, I was involved in experiments to determine whether any agonistic activity could be observed upon *in vivo* administration of the 4D11G4PE antibody. In particular, I was involved in experiments to determine what, if any, agonistic activity arose from administration of 100 mg/kg of the antibody in cynomolgus monkeys. These experiments and their results are summarized below.

7. Phosphate buffered saline (PBS) alone (tests #1 and #2) or combined with 100 mg/kg of 4D11G4PE (tests #3-#5) was administered intravenously to male cynomolgus monkeys for four weeks, once weekly. Blood was drawn from the femoral vein of the monkeys at indicated time points and was analyzed for IL-12 or IFNγ by ELISA.

8. The results are summarized in the following tables. Further details of the experimental methodology and results are set out in an attachment to this declaration.

Table 1. ***Serum IL-12 concentrations upon administration of 4D11G4PE***

Test no.	Before administration	24 h after 1st administration	24 h after 4th administration	7 d after 4th administration
#1	BLOQ	BLOQ	BLOQ	43.1
#2	41.9	13.7	19.4	29.0
#3	BLOQ	BLOQ	15.7	10.4
#4	128.7	82.3	23.6	93.6
#5	58.1	25.3	BLOQ	BLOQ
BLOQ (below the limit of quantification)				

Table 2. ***Serum IFNγ concentrations upon administration of 4D11G4PE***

Test no.	Before administration	24 h after 1st administration	24 h after 4th administration	7 d after 4th administration
#1	47.7	BLOQ	BLOQ	27.1
#2	BLOQ	BLOQ	BLOQ	42.1
#3	BLOQ	BLOQ	BLOQ	BLOQ
#4	4.3	72.7	18.2	34.3
#5	85.4	102.7	64.3	69.3
BLOQ (below the limit of quantification)				

9. These data demonstrate that the 4D11G4PE antibody shows not simply a reduction in but rather a negation of agonistic activity at a concentration of 100 mg/kg. The nominal variations or apparent "increases" in value are biologically insignificant.

10. I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 2010. 10. 15 By _____

Dr. Nobuaki Takahashi